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In re Application of:) TECH CENTER 1600/2900
PHILLIPS, NIGEL C.)
FILION, MARIO C.)
Serial No.: 09/857,332) Art Unit: 1635
Filed: JUNE 4, 2001) Examiner: J. Eric Angell
For: CHEMOTHERAPEUTIC)
COMPOSITION AND METHOD)

DECLARATION OF MARIO C. FILION, PH.D. UNDER 37 C.F.R. §1.132

I, Mario C. Filion, do hereby declare:

1. I am currently Head of Biomedical Research of Bioniche Life Sciences Inc., of Montreal, Quebec, Canada. I earned a B.Sc. degree in Molecular Biology in 1990 at Département de Biologie, Université du Québec à Montréal, Canada. I earned a M.Sc. degree in Molecular Biology in 1992 at Faculté des Etudes Supérieures, Université de Montréal, Canada. I earned the Ph.D. degree in Microbiology and Immunology in 1995 at Faculté de Médecine, Université de Montréal, Canada. In 1995-1998, I conducted post-doctoral studies at Neuro-Immuno-Endocrinology Unit, Institut Pasteur, Paris, France, and at the Faculté de Pharmacie, Université de Montréal, Montréal, Canada. In 1999-2000, I served as an Invited Professor at Faculté de Pharmacie, Université Laval, Québec, Canada. I am author or co-author of approximately fourteen articles in peer-reviewed scientific journals.

2. I am a named inventor on U.S. Patent Application Serial No. 09/857,332, entitled "Chemotherapeutic Composition and Method." I am familiar with the application and the rejections in the Office Action issued by the Examiner on December 17, 2002, in particular, the rejection of Claims 33-64 under 35 U.S.C. § 112, first paragraph.

3. The following experiments were performed under my supervision.

a. Inhibition of Jurkat T cell leukemia and EL-4 T-cell lymphoma cellular proliferation by combinations of chemotherapeutic drugs and MCC

Human Jurkat T cell leukemia cells and murine EL-4 T cell lymphoma cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were cultivated as suspension cultures in RPMI 1640 cell culture medium supplemented with 10% heat-inactivated fetal calf serum and containing 50 μ g/ml gentamycin sulfate (tissue culture medium: all from Sigma-Aldrich Canada, Oakville, Ontario). Cells were harvested by centrifugation (150 \times g for 15 minutes at 4°C), and resuspended in tissue culture medium to give a concentration of 1×10^5 cells/ml. Aliquots of this suspension (100 μ l each) were placed in wells of 96-well tissue culture plates. The chemotherapeutic drugs cytosine arabinoside, daunorubicin, and mitoxantrone were obtained from Sigma-Aldrich Canada (Oakville, Ontario). The chemotherapeutic drugs were dissolved in tissue culture medium, sterile filtered, diluted, and added to a sterile suspension of MCC in tissue culture medium (0.1 μ g/ml) to give the final drug concentrations of 0.001-1.0 μ g/ml. 100 μ l drug/MCC combination were then added to the cells, and incubation was carried out for 48 hours at 37°C in an atmosphere of 5.0% CO₂/95% air. At the end of the incubation, inhibition of cellular proliferation was determined by dimethylthiazoldiphenyltetrazolium (MTT) reduction (Mosman *et al.*, *Journ. Immunol. Methods* 65:55, 1983). The potency of the chemotherapeutic agent plus MCC was compared to the chemotherapeutic agent alone using software Pharm/PCS version 4.2 (Computer Associates, Philadelphia, PA, USA).

The concentration of MCC used in these studies was determined to give between 5-10% inhibition of proliferation in the absence of chemotherapeutic agents.

The concentrations of chemotherapeutic drugs used (0.001-1.0 $\mu\text{g/ml}$) were determined to give between 10 and 90% inhibition of cellular proliferation. The potency of the chemotherapeutic agents in combination MCC relative to the chemotherapeutic agents alone is shown in Table 1. The results show that the addition of sub-optimal amounts of MCC to cytosine arabinoside, daunorubicin or mitoxantrone significantly increased the potency of these drugs when compared to cytosine arabinoside, daunorubicin or mitoxantrone alone.

Table 1
MCC enhances the potency of chemotherapeutic agents

| Chemotherapeutic agent | Relative potency (95% Confidence Limits) | | | |
|------------------------|--|----------------|----------------------|----------------|
| | Jurkat T cell leukemia | | EL-4 T cell lymphoma | |
| | No MCC | +MCC | No MCC | +MCC |
| Cytosine arabinoside | 1.0 | 8.4 (1.9-55) | 1.0 | 5.3 (2.0-16.9) |
| Daunorubicin | 1.0 | 4.0 (0.6-81.0) | 1.0 | 6.0 (2.0-19.0) |
| Mitoxantrone | 1.0 | 4.4 (1.5-32) | 1.0 | 7.2 (2.0-45 0) |

These results demonstrate that the combination of MCC with chemotherapeutic agents significantly increased their potency against leukemia and lymphoma cells.

b. MCC potentiation of the antiproliferative activity of mitoxantrone on prostate LNCaP cancer cells

The prostate LNCaP cancer cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). LNCaP cells were cultured in RPMI 1640 cell culture medium containing 10% heat-inactivated fetal bovine serum and 50 $\mu\text{g/ml}$ gentamycin (all from Sigma-Aldrich Canada, Oakville, Ontario) at 37°C in an atmosphere of 5.0% CO_2 /95% air. LNCaP cells were incubated in individual wells of 24-well plates at 2.0×10^5 cells/ml for 72 hours with saline, 0.1